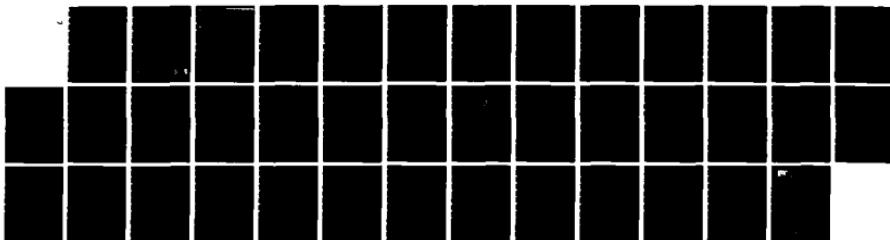


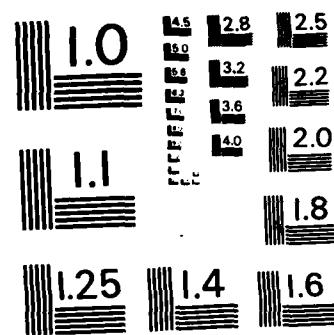
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PHLEBOTOMUS FEVER VIRUSES IN PANAMA (U)

Final Report

Jack W. Millar

(For the period 1 September 1980 to 31 August 1981)

August 1981

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701

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Gorgas Memorial Laboratory  
Panama, Republic of Panama

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The epidemiology of Phlebotomus fever group viruses was studied in Panama. The project was subdivided into component sections: the incidence of Phlebotomus fever group viruses among US military personnel assigned to Panama; a serologic survey and experimental infections of sylvatic vertebrates; an analysis of antigenic variation among strains of Chagres and Punta Toro viruses; laboratory rearing of phlebotomine sandflies; and Phlebotomus fever group viruses. The plaque reduction neutralization (PRN) test (constant virus: serum dilution) was used for all serologic testing. Serologic surveys included Chagres (CHG) (Continued)		

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20. (Continued) and Punta Toro (PT) viruses of the Phlebotomus fever group. Prototype viruses of both CHG and PT viruses or low passage local isolates shown to be antigenically very similar to the prototype strains were used in all sero-surveys. Results of the study of these viruses among US soldiers in Panama found that both CHG and PT viruses were actively transmitted to US military personnel in Panama during 1980; that the incidence of PT viruses was approximately two-fold greater than the incidence of CHG viruses; that military companies which frequently engaged in field training exercises (infantry and combat support companies) had a higher incidence rate than did those companies which spent less time in field training; and that the incidence of infection among troops assigned to the Atlantic side of Panama was slightly greater than among those assigned to the Pacific side.

The serologic survey of residents of rural Panama found that: antibody to PT virus was present among residents of all areas of Panama tested, with the exception of Las Perlas Islands; antibody to CHG virus was restricted to residents of eastern and central Panama and virtually absent from western Panama; the antibody prevalence rate for PT virus was about two-fold higher than that for CHG virus; and the highest antibody prevalence rates to both PT and CHG viruses were found among residents of Darien Province in eastern Panama.

The serologic survey of Panamanian cattle was based on the close antigenic relationship previously shown between PT virus and Rift Valley fever (RVF) virus, an important human and veterinary pathogen found in Africa. The objective of this study was to determine the prevalence of antibody to both viruses as a possible indicator of cross protection between the two viruses or undetected diseases due to one or both agents. This study determined that neutralizing antibody to PT virus was found in sera from cattle reared in several areas of Panama, with highest rates at about 7% in Coclé Province and a country-wide rate of about 4%. No consistent pattern of reaction was detected when cattle sera which contained anti-PT neutralizing antibody were reacted against RVF virus.

The objective of the serologic survey and experimental infections of sylvatic vertebrates was to identify those species of vertebrates most likely to serve as amplifying hosts of Phlebotomus fever group viruses. Results indicated that primates, porcupines, armadillos and Liomys rodents were most frequently found with anti-PT virus antibody; antibody to CHG virus was found less frequently than was antibody to PT virus, with Liomys rodents, porcupines and primate sera found to contain anti-CHG virus antibody; antibody prevalence rates among approximately 1000 sylvatic mammal sera tested was 10% of PT virus and 1% for CHG virus; and experimental infection of Liomys rodents indicated that viremia followed infection with PT virus but not with CHG virus.

Analysis of antigenic variation among strains of CHG and PT viruses found significant biological differences between strains of CHG virus recovered from similar geographic areas as shown by hamster mortality following experimental infection. Significant antigenic differences also exist between strains of CHG virus and preliminary results indicate that antigenic and biologic differences correlate well and support two distinct subtypes of CHG-like viruses. Antigenic, biologic and molecular differences were also noted among strains of PT viruses, but not as great as those seen with CHG viruses.

Results of transmission attempts of PT and CHG viruses were too fragmentary to allow meaningful conclusions to be drawn, with the exception that PT virus was clearly transovarially transmitted by four species of sandflies.

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Summary

The epidemiology of Phlebotomus fever group viruses was studied in Panama. The project was subdivided into component sections: the incidence of Phlebotomus fever group viruses among US military personnel assigned to Panama; a serologic survey of cattle in Panama; a serologic survey and experimental infections of sylvatic vertebrates; an analysis of antigenic variation among strains of Chagres and Punta Toro viruses; laboratory rearing of phlebotomine sandflies; and Phlebotomus fever group viruses. The plaque reduction neutralization (PRN) test (constant virus: serum dilution) was used for all serologic testing. Serologic surveys included Chagres (CHG) and Punta Toro (PT) viruses of the Phlebotomus fever group. Prototype viruses of both CHG and PT viruses or low passage local isolates shown to be antigenically very similar to the prototype strains were used in all sero-surveys. Results of the study of these viruses among US soldiers in Panama found that both CHG and PT viruses were actively transmitted to US military personnel in Panama during 1980; that the incidence of PT viruses was approximately two-fold greater than the incidence of CHG viruses; that military companies which frequently engaged in field training exercises (infantry and combat support companies) had a higher incidence rate than did those companies which spent less time in field training; and that the incidence of infection among troops assigned to the Atlantic side of Panama was slightly greater than among those assigned to the Pacific side.

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## Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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I. Incidence of Phlebotomus fever group viruses among U. S. military personnel assigned to Panama.

A. Statement of problem

The objective of this study was to determine the incidence of Phlebotomus fever (PF) group viruses among U.S. military personnel assigned to Panama.

B. Background

Four American military units are located in Panama which frequently engage in field training exercises. These are the 3rd Battalion, 5th Infantry stationed at Fort Kobbe, and the 4th Battalion 20th Infantry stationed at Fort Clayton, both of which are on the Pacific side of Panama. On the Atlantic side are the 4th Battalion, 10th Infantry stationed at Fort Davis, and the 3rd Battalion, 7th Special Forces Group stationed at Fort Gulick. Each battalion contains approximately 7-800 men, except the Special Forces Group at Fort Gulick, which has slightly over 300 men.

Within each battalion are 3 infantry companies (A, B, and C) a combat support company and a headquarters company. The infantry companies and the combat support company are engaged in training exercises or maneuvers which take them into the forest almost every week, thus they represent the segment with the highest rate of field exposure among U.S. military stationed in Panama. The headquarters companies also enter the field routinely, but not as frequently as the other companies. Several support units are located on each post which have little or no field exposure. Consequently a gradation exists in the degree of field exposure within this population.

Personnel assigned to these units remain in Panama for various lengths of time. Generally an individual not accompanied by dependents has a tour of duty of 24 months. Those accompanied usually have a 36 month tour. It is not uncommon, however, to have people rotate out after as few as 12 months in the unit.

Field training exercises are conducted in essentially undisturbed tropical forest which extend 5 miles inland on either side of Panama Canal. Those companies which frequently engage in training exercises spend from 10-30% of their time on an annual basis, in the field. Typically field training exercises last 3 days and 2 nights, but longer exercises are not uncommon.

### C. Approach to problem

A total of 2222 sera from U.S. military personnel assigned to Panama was available for testing. These sera were from individuals with various length of time in Panama, from less than 30 days to more than 3 years in country. These sera were tested by plaque reduction neutralization (PRN) test for neutralizing (N) antibody to both Chagres (CHG) and Punta Toro (PT) viruses, the 2 PF group viruses in Panama known to cause human illness. Since both viruses are known only from New World tropics, we assumed that U.S. soldiers arrived in Panama seronegative. Thus, any antibody-positive individuals were most likely infected while in Panama. Since the exact date of arrival in Panama was known for each of these soldiers, we were able to calculate the incidence of infections in this well defined susceptible adult population.

### D. Results and discussion

Sera from 2222 soldiers assigned to the 4 military units under study examined by PRN tests for antibody to both CHG and PT viruses. Included in these are 397 sera from individuals with less than 30 days in country. Sera from these newly arrived persons have been uniformly negative for N antibody to both viruses and may serve as pre-exposure controls in support of our assumption that all U.S. military arrive seronegative.

Records for the remaining 1,825 individuals with greater than 30 days in country were reviewed to determine each individual's length of time in Panama. Total man-years of exposure were calculated for each company and the ratio of the number of sera tested to the number of man-years of exposure was calculated. The ratio is approximately 1:1 for each unit; consequently, the rates presented are essentially annual incidence rates.

An overall annual incident rate of 0.8/100 was found for Punta Toro virus (Table 1). When analysed by company, the highest incidence rates were found among the infantry and combat support companies with rates of 1.3 and 1.4/100 respectively. Incidence rates for the headquarters and other support units with less field exposure were 0.3 and 0.2/100 respectively.

The overall incidence of Chagres virus was 0.3/100, or about half that detected for Punta Toro virus (Table 1). All seropositive individuals were from the infantry and combat support companies with rates of 0.5 and 0.7/100 respectively.

No seropositive individuals were from the headquarters or other support groups.

When compared by site of assignment, those stationed on the Atlantic side of Panama had a slightly higher rate of infection for both viruses than did those stationed on the Pacific side, 0.3 versus 0.2/100 for CHG virus and 1.0 versus 0.6 for Punta Toro virus (Table 2).

In addition to the serological investigations, clinical samples were referred to us by troop medical clinics which supported these units for diagnostic assistance in attempts to isolate virus from acutely ill febrile patients. From 56 sera tested, 4 viruses were isolated; 1 PT, 2 CHG and 1 strain of Venezuelan equine encephalitis (VEE) virus, enzootic subtype 3880.

The recovery of VEE virus from a febrile soldier was of special interest to us since this virus is of recognized military importance yet maintained in an enzootic cycle quite distinct from that proposed for PT group viruses. We seized this virus recovery as an opportunity to compare the incidence of these 2 ecologically distinct arbovirus groups among American soldiers assigned to Panama. We made the assumption, as with PT and CHG viruses, that all US military personnel arrive in Panama lacking antibody to enzootic VEE virus. This assumption allowed us to calculate incidence rates for VEE virus, as was done for PT and CHG viruses, based on seropositivity and length of time in Panama. While this assumption for VEE virus is somewhat more tenuous than that for CHG and PT viruses due to the possibility of exposure to epizootic VEE in the U.S. during the 1971 outbreak, we felt that the likelihood of an individual arriving with anti-VEE antibody was still quite small when compared to his potential for exposure here. Results of testing of the sera from newly arrived soldiers for antibody to enzootic VEE has been negative throughout, thus supporting this interpretation.

We have completed testing all 2222 sera for N antibody to enzootic VEE virus using the strain isolated from our febrile soldier. Results of this study have found annual incidence rates by type of company as follows: Infantry Companies, 0.5/100; Combat Support Companies, 1.0/100; and other support companies, 0.3/100. The overall annual incidence rate for enzootic VEE virus was 0.5/100, which is comparable to those previously reported for CHG and PT viruses of 0.3 and 0.8/100, respectively (Table 3).

To summarize these results, we can conclude that the incidence of PT virus is about 2-fold greater among these troops than is CHG virus. Second, as expected, companies which frequently engage in field exercises have a greater incidence of infection than those who are less frequently exposed. Finally, the incidence of infection among troops assigned to the Atlantic side of the isthmus is slightly greater than that among those assigned to the Pacific side.

With the normal strength of a company about 150 people, we might then expect 1 case of CHG and 2 cases of PT infection per company per year under current training regimes for frequently exposed companies. For companies less frequently exposed, we might expect a single case of PT virus per company per year and very few if any CHG virus infections.

The low incidence of Phlebotomus fever group viruses may perhaps be explained by the training regime of these troops. Most field exercises are of limited duration, usually less than one week. Also, the use of insect repellent, bed netting and the like are stressed among troops during these training exercises. The net result is that the probability of an individual becoming infected is quite low. If a person is infected, he almost never remains in the field, consequently no amplification of the virus among feeding vectors takes place and under these settings man is effectively a dead-end host. In essence troops are exposed only to the enzootic transmission cycle of these viruses and unless an epizootic is in progress, few vectors would be expected to be infected.

A different pattern of transmission might be expected for large groups of migrant workers, settlers or the military under hostile conditions. These groups would remain in the field for longer periods. The associated greater duration of exposure and relaxing of personal protective measures could result in increased rates of infection. Were febrile patients exposed to feeding vectors, amplification of the virus prevalence among vectors might occur. Such conditions would obviously be ideal for increased rates of transmission and perhaps outbreaks.

#### E. Conclusions

1. Both PT and CHG viruses were actively transmitted to U.S. military personnel in Panama during 1980.
2. The incidence of PT virus was approximately 2-fold greater than the incidence of CHG virus among U.S. troops.

Table 1. Comparison of annual incidence rates of Punta Toro and Chagres viruses among U.S. military personnel by type of company and average annual number of nights field exposure (per cent of year); Panama, 1980.

Company	Ave. # Nights Exposure (%)	# Sera Tested	Punta Toro Virus		Chagres Virus	
			No.	Pos.	Rate*	No. Pos.
Infantry	89.2 (24%)	630	8	1.3	3	0.5
Combat Support	65.6 (18%)	279	4	1.4	2	0.7
Headquarters	35.3 (10%)	354	1	0.3	0	-
Others	ND**	562	1	0.2	0	-
TOTAL		1825	14	0.8	5	0.3

\* Annual incidence rate/100

\*\* ND = not done

Table 2. Comparison of annual incidence rates for Punta Toro and Chagres viruses among 1825 U.S. military personnel by site of assignment, Fort and type of company; Panama, 1980.

Site	Fort	Companies	Punta Toro Virus			Chagres Virus		
			No. Tested	No. Pos.	Rate*	No. Pos.	Rate*	
Atlantic	Davis	INF/CS	347	4	1.1	2	0.5	
		HQ/Others	203	1	0.5	0	—	
Gulick		INF/CS	23	1	3.3	0	—	
		HQ/Others	5	0	—	0	—	
Atlantic Totals		INF/CS	370	5	1.4	2	0.5	
		HQ/Others	208	1	0.5	0	—	
		OVERALL	578	6	1.0	2	0.3	
Pacific	Clayton	INF/CS	380	4	1.1	2	0.5	
		HQ/Others	587	1	0.2	0	—	
		OVERALL	121	3	1.7	1	0.6	
Pacific Totals		INF/CS	539	7	1.3	3	0.6	
		HQ/Others	708	1	0.1	0	—	
		OVERALL	1247	8	0.6	3	0.2	

\* Annual incidence rate per 100

Table 3. Annual incidence rates of Venezuelan equine encephalitis virus (enzootic subtype 3880) among U.S. military personnel by type of company; Panama, 1980.

Company	No. Tested	No. Pos.	Rate*
Infantry	630	3	0.5
Combat Support	279	3	1.0
Headquarters	354	1	0.3
Others	562	2	0.3
Totals	1825	9	0.5

\* Annual incidence rate per 100

3. Companies which frequently engaged in field training exercises (Infantry and combat support companies) had a higher incidence rate than did those companies which spent less time in field training (Headquarters and other companies).

4. The incidence of infection among troops assigned to the Atlantic side of Panama was slightly greater than among those troops assigned to the Pacific side.

## II. Serological survey of rural Panama

### A. Statement of problem

The objective of this project was to determine the distribution of PT and CHG viruses throughout Panama by determining the N antibody prevalence rates to these viruses among human residents of Panama, excluding Panama City.

### B. Background

A sero-survey of Panama was conducted by the Panamanian government in 1978 and these sera were available to us for testing. The survey was a random one percent sample of the Panamanian population older than one year. Nine provinces comprise the Republic of Panama. Provinces are divided into Districts which for census purposes are further subdivided into segments. Each segment contains 30-40 households. The Epidemiology Division, Ministry of Health picked the survey sample by randomly selecting census segments within each district, then randomly selecting households from each segment. The number of segments and households was determined so that by bleeding all residents of selected households one percent of the district population would be sampled. The only constraint placed on random selection was that at least one rural and one urban segment had to be sampled in each district. Exact details of sampling were determined prior to the survey.

The Provinces of Darien and San Blas were not included in the 1% random sample. For Darien Province, sera collected from various localities in the Province between approximately 1970 and 1980 were examined. Selected sera from other Provinces collected since 1975 were also examined. These sera were generally from residents of rural areas and thus would be expected to be at greater risk of exposure to sandfly vectors.

### C. Approach to problem

Approximately 6000 sera were available for testing for antibody to PF group viruses. These sera were tested by PRN

tests for antibody to CHG and PT viruses using a constant virus dose (25-100 plaque forming units) and a 1:4 serum dilution on Vero cells grown in 96 well panels, 2 well/serum.

#### D. Results and discussion

The 1% random sample of central and western Panama was completed by the Panamanian government during 1978. These sera were used as the basis for our study to determine the distribution and prevalence of antibody to CHG and PT viruses among residents of rural Panama. This sample included residents of both rural and urban setting outside of Panama city, and thus contained many sera from persons with little or no exposure to vectors of sandfly fever. In addition to sera from the 1% random sample, several sera were available from studies of selected rural populations which had substantially greater potential for exposure to insect vectors.

Neutralizing antibody prevalence rates to PT virus among sera from the random sample were 5% or less for all provinces tested except Chiriqui. Rates were substantially higher in samples collected from selected rural communities, ranging from a high of 34% among sera from the Darien to 8% among sera from the Changuinola Valley in Bocas del Toro Province (Figure 1).

Antibody prevalence rates to CHG virus were lower than those to PT virus in all instances. The rate was 2% or less for the provinces in Central Panama, and antibody to CHG virus was virtually absent among residents of western Panama (Figure 2). The highest rate detected was among selected sera from the Darien at 17%. A summary of these antibody prevalence rates is presented in Table 4.

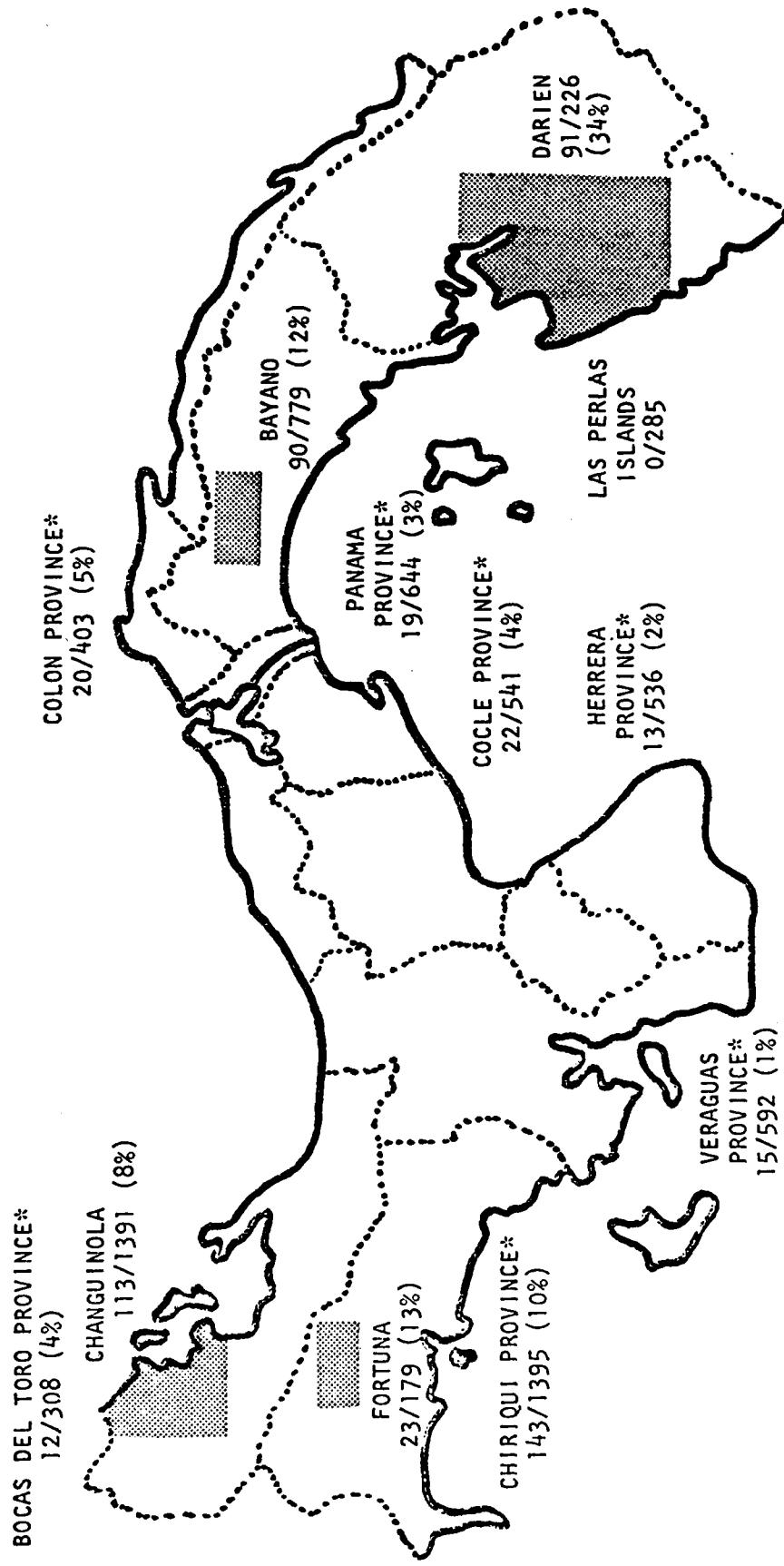
#### E. Conclusions

1. Antibody to PT virus was found among residents of all areas of Panama tested, with the exception of Las Perlas Islands.
2. Antibody to CHG virus was restricted to residents of eastern and central Panama and virtually absent from western Panama.
3. The antibody prevalence rate for PT virus was about 2-fold higher than that for CHG virus.
4. Highest antibody prevalence rates to both PT and CHG viruses were found among residents of Darien Province in eastern Panama.

Table 4. Neutralizing antibody prevalence rates to Chagres and Punta Toro viruses among residents of Panama.

Province	CHAGRES		PUNTA TORO	
	<u>1% sample</u>	<u>Selected pops.</u>	<u>1% sample</u>	<u>Selected pops.</u>
Bocas del Toro	0/308 ( 0%)	3/1395 (<1%)	12/308 ( 4%)	113/1391 ( 8%)
Chiriqui	9/1640 (<1%)	-	143/1395 (10%)	23/179 (13%)
Cocle	10/541 ( 2%)	-	22/541 ( 4%)	-
Colon	3/390 (<1%)	-	20/403 ( 5%)	-
Darien	-	46/226 (17%)	-	91/226 (34%)
Herrera	9/552 ( 2%)	-	13/536 ( 2%)	-
Las Perlas Islands	-	0/285	-	0/285
Los Santos	2/525 (<1%)	-	16/529 ( 3%)	-
Panama	16/634 ( 3%)	-	19/644 ( 3%)	90/779 (12%)
Veraguas	6/592 ( 1%)	-	15/592 ( 3%)	-

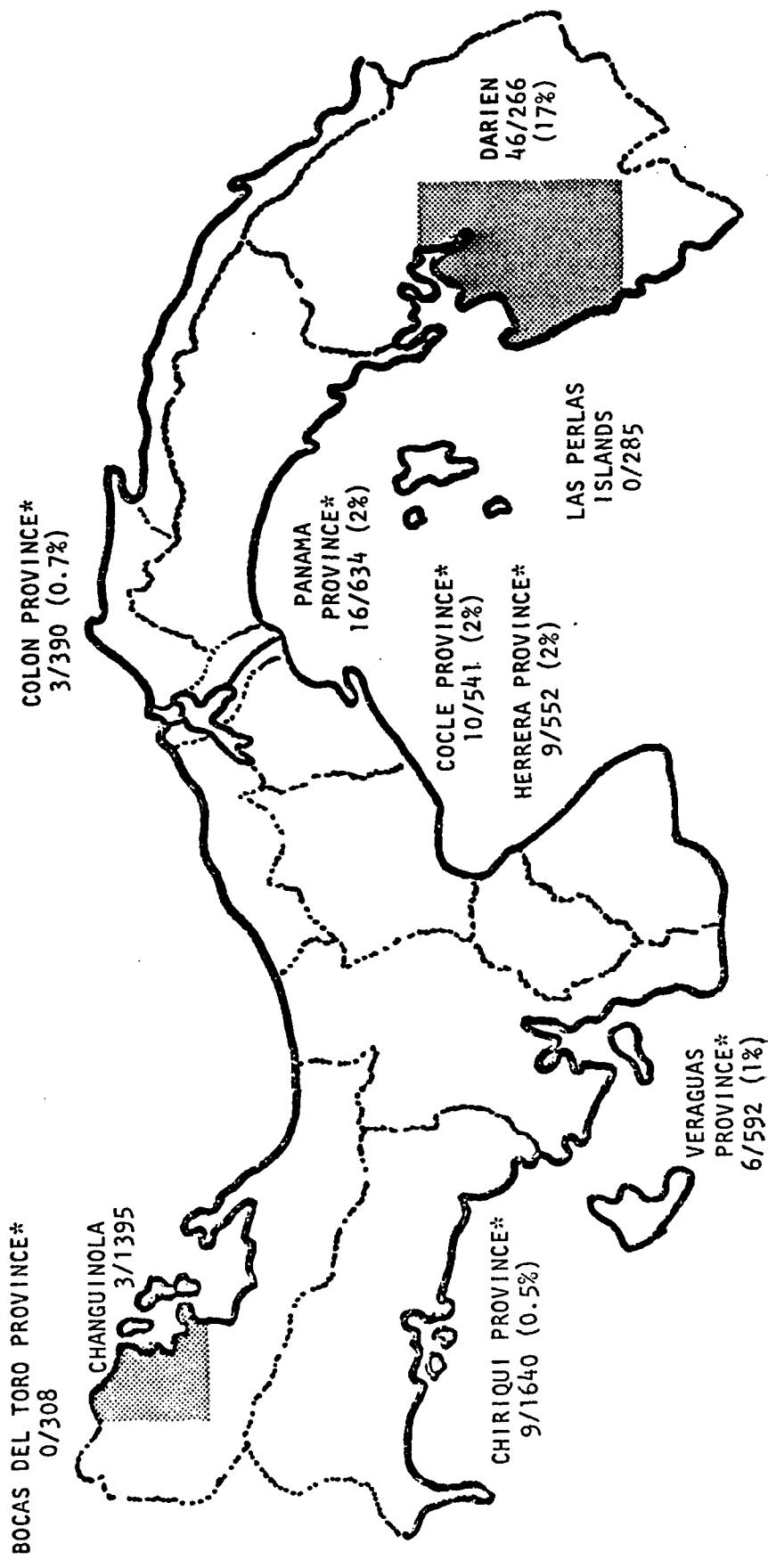
NEUTRALIZING ANTIBODY PREVALENCE RATES TO PUNTA TORO VIRUS AMONG  
RESIDENTS OF PANAMA BY PROVINCE OR AREA



\* SERA FROM 1% RANDOM SAMPLE OF PANAMA

Figure 1

NEUTRALIZING ANTIBODY PREVALENCE RATES TO CHAGRES VIRUS AMONG RESIDENTS OF PANAMA



III. Serologic survey of cattle (in conjunction with USAMRIID, Ft. Detrick, MD).

A. Statement of problem

The objective of this project was to determine the antibody prevalence rates to PT and Rift Valley Fever (RVF) viruses among cattle in Panama.

B. Background

Recent studies have demonstrated a close antigenic relationship between RVF and PT virus. RVF is a very important disease of domestic animals, especially cattle and sheep, as well as a human pathogen. The close antigenic similarity between the 2 viruses brings to mind questions of possible cross protection or undetected disease in domestic animals in Panama.

C. Approach to problem

Sera from cattle raised in various regions of Panama were collected at the time of slaughter and tested by the PRN test for antibody to PT virus. In Panama, cattle are slaughtered at age 3 to 5 years. While cattle are raised in many areas of the country, most are shipped to Panama City for slaughter. As a result, we were able to collect sera locally for testing from cattle reared throughout the country. The positive sera were titrated and a selection of both positive and negative sera were forwarded to USAMRIID for setting against RVF.

A killed RVF hemagglutination antigen was provided to us by USAMRIID to allow us to screen cattle sera by the hemagglutination inhibition (HI) test locally. Cattle sera so tested were acetone extracted prior to HI testing.

D. Results and discussion

A total of 423 cattle sera were collected and tested for neutralizing antibody to PT virus. These sera were from Chiriqui (16), Veraguas (34), Los Santos (316), Coclé (17), Panama (28) and Darien (12) provinces. Of these, 16 (3.7%) gave positive (>90% reduction) and additional 27 (6.3%) gave equivocal (80-89% reduction) results. Positive reactors were found in Chiriqui (1 of 16, 6%) Veraguas (3/34, 9%), Los Santos (11/316, 3%) and Coclé (1/17, 6%) provinces. Equivocal reactors were also restricted to these provinces.

Thirteen positive, 13 equivocal and 27 negative reactors were selected and forwarded to USAMRIID Fort Detrick, Maryland for testing against RVF virus. Using a 1:10 serum dilution and >90% reduction as positive, one serum was clearly positive (1:80-1:160 dilution gave 90% reduction) and an additional 4 sera were equivocal. Several additional sera showed reactivity at a 50% or 80% level of reduction, though the significance of these lesser reactions is presently unclear. The single positive reactor had given equivocal results when tested against PT virus. Of those equivocal to RVF, 2 had been positive and 2 had been equivocal when tested against PT virus. Consequently, a significant degree of cross-protection between RVF and PT viruses was not seen.

Since the positive serum and one of the 4 equivocal sera were collected from the same locality, Anton in Coclé province, an effort was made to collect additional sera from cattle in that area through an ongoing collaborative project between GML and the Ministry of Agriculture, Brucellosis surveillance campaign field office near Anton. We received 406 cattle sera from the Anton area, including 20 from cows which recently aborted. We were notified by the local veterinarian of these abortions, many of which were of near term calves, and advised that this number of cattle abortions was much greater than would normally be expected. Of the 20 sera from cows which had recently aborted, 2 (10%) had N antibody to PT virus and another 1 (5%) had antibody to RVF when tested by the hemagglutination inhibition (HI) technique. These results suggests that a Phlebovirus was probably not involved in the cattle abortion epizootic.

We have completed HI testing of 298 cattle sera from Anton for antibody to RVF virus. Only a single serum had HI antibody to RVF. A total of 406 sera have been tested by plaque reduction neutralization (PRN) tests for antibody to PT virus. Of these, 28 (7%) had anti-PT antibody.

Results of this subproject indicate that the RVF-like virus suspected of infecting cattle in Panama is either very uncommon or does not exist. The antigenic similarities reported earlier between RVF and PT viruses are not reflected in cross-protection studies when sera from cattle apparently naturally infected with PT virus are challenged with RVF virus. One might expect then, that should RVF virus be introduced into Panama, the presence of anti-PT antibody would not significantly affect the course of RVF virus transmission.

### E. Conclusions

1. Neutralizing antibody to PT virus was found in sera from cattle reared in several regions of Panama with a country-wide antibody prevalence rate of about 4% (16/423).
2. Highest antibody prevalence rates to PT virus were found in cattle reared around Anton, Coclé Province at about 7% (28/406).
3. No consistent pattern of reaction was detected when cattle sera which contained anti-PT N antibody was reacted in either the PRN or HI test against RVF virus. This implies that anti-PT antibody would not cross protect if challenged with RVF virus.

## **IV. Serologic survey and experimental infections of sylvatic vertebrates.**

### A. Statement of problem

The objectives of this project were to initially identify those species of vertebrates most likely to serve as amplifying hosts of PF group viruses through a serologic survey. Once specific hosts were identified, attempts were made to experimentally infect selected individuals of those species in order to determine which species, that when infected produced a viremia of sufficient titer to infect feeding sandfly vectors.

### B. Background

Little information has been gathered to identify the vertebrate hosts of PF group viruses. Indeed, some have questioned the necessity of vertebrate hosts at all in the maintenance of PF group viruses in nature in view of transovarial transmission of these viruses. While some form of horizontal transmission is necessary to replace virus lost during vertical transmission, the exact source of this replenishment remains unknown. This facet of our study was designed to help to identify potential vertebrate sources of this virus amplification in nature.

### C. Approach to problem

Results of PRN tests on sylvatic vertebrate sera held in the serum bank were used to identify those species most likely to be natural hosts of PF groups viruses in nature. Members of selected species were then collected or purchased and brought to the laboratory to be experimentally infected. Indi-

viduals lacking N antibody were inoculated subcutaneously with 100-1000 pfu of diluted virus. Infected animals were bled daily for 7 days and whole blood immediately diluted 1:10 in Vero cell culture maintenance medium. Following the final bleeding, each sample was further diluted  $10^{-2}$  thru  $10^{-5}$  and assayed together with the original inoculum by the direct plaque method on Vero cells grown in 96 well panels.

Experimental animals were held until at least day 30 post inoculation when they were again bled and their sera tested for N antibody to the homologous virus or for evidence of persistent infection.

#### D. Results and discussion

Sera from 1025 sylvatic vertebrates have been tested by PRN tests for antibody to PT virus. Results are presented in Table 5. Anti-PT antibody was found in : Oryzomys (1 positive/11 tested); Sigmodon (1/68); Liomys (2/50); Coendou (8/45); Caluromys (1/12); Cebus (4/37); Ateles (8/51); Aotus (4/63); Alouatta (33/177); Ateles (33/55) and Dasyprocta (5/36).

Sera from 977 sylvatic vertebrates have been tested by PRN test for antibody to CHG virus. Results of these tests are also shown in Table 5. Anti-CHG antibody was found less commonly than anti-PT antibody. Positive animals included: Liomys (3/45); Coendou (2/37); Ateles (1/45); Aotus (1/63); Alouatta (31/63) and domestic pigs (2/51).

We have attempted to experimentally infect 9 Liomys adspersus in the laboratory, 6 with CHG virus and 3 with PT virus. Of the 6 Liomys inoculated with CHG virus, none produced a detectable viremia. Of 3 Liomys inoculated with PT virus, 2 resulted in a detectable viremia. One had a viremia of approximately  $1.1 \times 10^6$  pfu/ml on day 5 post-inoculation. It was negative for viremia on days 4 and 7 and not bled on day 6. The second animal had a viremia of approximately  $1.9 \times 10^6$  pfu/ml on day 7. It was negative on day 5 and not bled on days 6 and 8 or beyond.

Cross challenge experiments were not attempted.

Dr. Howard Christensen of GML has recently completed blood meal determinations on four species of Lutzomyia which are thought to be vectors of PF group viruses: Lu. ypsilon; Lu. trapidoi; Lu. gomezi and Lu. panamensis. Identification of blood meal sources provides an additional marker to identify likely vertebrate amplifying hosts of PF group viruses in nature. Dr. Christensen's results indicated substantial feeding

Table 5. Results of plaque reduction neutralization tests for antibody to Punta Toro and Chagres viruses among sylvatic vertebrates captured in Panama.

Scientific Name (Common Name)	PUNTA TORO			CHAGRES		
	Total # tested	# Pos. (%)	Total # tested	# Pos. (%)	Total # tested	# Pos. (%)
<i>Oryzomys talamancae</i> (Rice rat)	11	1 ( 9)	7	7	—	—
<i>Zygodontomys</i> (Cane rat)	7	—	—	—	6	—
<i>Sigmodon hispidus</i> (Cotton rat)	68	1 ( 1)	—	—	43	—
<i>Liomys adspersus</i> (Spiny pocket mouse)	50	2 ( 4)	—	—	45	3 ( 7)
<i>Proechimys</i> (Spiny rat)	185	—	—	—	150	—
<i>Hoplomys gymnurus</i> (Spiny rat)	36	—	—	—	35	—
<i>Diplomys darlingi</i> (Climbing spiny rat)	11	—	—	—	9	—
<i>Oryzomys albicularis</i>	7	—	—	—	11	—
<i>Cricetus cricetus</i>	48	—	—	—	88	—
<i>Rattus</i>	3	—	—	—	—	—
<i>Tylomys nudicaudatus</i>	1	—	—	—	1	—
<i>Coendou rothschildi</i> (Porcupine)	45	8 (18)	—	—	37	2 (5)
<i>Caluromys derbianus</i> (Woolly opossum)	12	1 ( 8)	—	—	13	—
<i>Philander opossum</i> (Four-eyed opossum)	—	—	—	—	8	—
<i>Metachirus nudicaudatus</i> (Brown masked opossum)	9	—	—	—	15	—
<i>Didelphis marsupialis</i> (Common opossum)	15	—	—	—	10	—
<i>Cebus capucinus</i> (White face monkey)	12	—	—	—	38	—
<i>Ateles geoffroyi</i> (Spider monkey)	37	4 (11)	—	—	45	1 (2)
<i>Aotus trivirgatus</i> (Night monkey)	51	8 (16)	—	—	63	1 (2)
<i>Alouatta villosa</i> (Howler monkey)	63	4 ( 6)	—	—	163	3 (2)
<i>Ateles fusciceps</i> (Black spider monkey)	177	33 (19)	33 (60)	56	—	—
<i>Saguinus geoffroyi</i> (Marmoset)	55	—	—	—	19	—
<i>Bradypterus griseus</i> (3-toed sloth)	21	—	—	—	11	—
<i>Choloepus hoffmanni</i> (2-toed sloth)	8	—	—	—	14	—
<i>Dasyurus novemcinctus</i> (9-banded armadillo)	17	—	5 (14)	39	—	—
<i>Domestic pig</i>	36	—	40	—	51	2 (4)
<b>TOTAL.....</b>	<b>1025</b>	<b>100 (10)</b>	<b>977</b>	<b>977</b>	<b>12 (1)</b>	

by Lutzomyia on sloths, rabbits, porcupines, primates and opossums. Blood feeding rates on rodents, aside from porcupines, were generally less than 1%.

Compilation of the serological results with Dr. Christensen's blood meal identifications suggests that the vertebrate species most likely to serve as amplifying hosts of PF group viruses are porcupines or primates. Unfortunately we were unable to acquire either for experimental infection studies. While sloths were uniformly serologically negative, the high rate of feeding upon them by Lutzomyia (to 65% in Lu. trapidoi) indicates that experimental infections of these hosts would also be justified.

#### E. Conclusions

1. Serological results indicate that primates, porcupines, armadillos and Liomys rodents were most frequently found with anti-PT virus antibody.

2. Antibody to CHG virus was found less frequently than was antibody to PT virus, with Liomys rodents, porcupines and primate sera found to contain anti-CHG virus antibody.

3. The overall antibody prevalence rate among approximately 1000 sylvatic mammal sera tested was 10% for PT virus and 1% for CHG virus.

4. Experimental infection of Liomys rodents indicated that viremia followed infection with PT virus but not with CHG virus.

#### V. Antigenic variation among strains of Chagres and Punta Toro viruses.

##### A. Statement of problem

The objective of this project was to antigenically characterize recently isolated, low passage strains of CHG and CHG-like viruses and PT and PT-like viruses. Those strains showing greatest antigenic variation were selected for further testing at the molecular level in an attempt to correlate molecular differences with antigenic variation.

##### B. Background

Several isolations of PF group viruses have been made in recent years from insects, animal tissues and human blood

collected in Panama. Most common among these has been isolates similar or identical to either CHG virus or PT virus. Many of these have been made from material collected at the Bayano field study site and thus represent samples from the same ecosystem. Although these viruses are from similar habitats, preliminary characterization indicates a considerable degree of antigenic variation between isolates.

#### C. Approach to problem

Five strains of CHG and CHG-like viruses and 6 of PT and PT-like viruses were selected for study. Each strain tested was inoculated subcutaneously into hamsters. Surviving hamsters were exsanguinated on day 30 post inoculation. Each immune serum was tested in constant virus-serum dilution PRN tests against the homologous virus, the remaining strains and the appropriate prototype virus.

Strains which show the greatest antigenic differences were forwarded to Dr. David Bishop (University of Alabama) to be tested in 2-dimensional electrophoretic oligonucleotide mapping experiments in an attempt to identify the molecular basis for the observed antigenic variation.

#### D. Results and discussion

Punta Toro viruses: 6 isolates of PT virus were selected for testing. These included the prototype strain isolated from a man infected in Fort Sherman, the former Canal Zone; an isolate from a soldier station at Fort Kobbe, also in the former Canal Zone in 1979; an isolate from man infected in the Darien Province in 1972; an isolate from a sentinel hamster infected in the Bayano River Basin, Panama Province in 1976; an isolate from a pool of sandflies (Lutzomyia sanguinaria) collected in the Bayano in 1976; and an isolate from a sentinel hamster infected in the Bayano in 1980.

Results shown in Table 6 indicate that of the 6 PT strains inoculated into hamsters, 4 killed all hamsters at all titers sufficient to initiate infection. Of the 2 remaining strains, both killed some hamsters inoculated, but some infected hamsters did survive and yielded immune sera. We then infected adult white rats with all 6 PT virus strains and harvested immune sera from them. In general, rat anti-PT antibody was very low titered, ranging from 1:4 to 1:128 homologous titers with most in the 1:16 - 1:32 range. Using these reagents, we completed the cross neutralization tests. As high as 32-fold differences have been found with some virus-antibody combina-

Table 6. Hamster survival rates following subcutaneous inoculation with various strains of Punta Toro virus and results of cross Plaque reduction neutralization tests using immune sera (30 day harvest) from surviving hamsters or rats (\*\*).

<u>ViruS</u>	<u>HAMSTER</u> ( <u>Sur/Inoc</u> )	<u>1</u>		<u>2</u>		<u>3</u>		<u>4</u>		<u>5</u>		<u>6</u>	
		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
1. Prototype	3/5			<u>128*</u>	13	64	24	58	32				
2. Human, Ft. Kobbe, 1979	0/14			109	<u>77**</u>	16	24	29	16				
3. Human, Darien, 1972	1/5			16	14	<u>32</u>	12	29	16				
4. Hamster, Bayano, 1976	0/4			16	14	32							
5. <u>Lutzomyia</u> , Bayano, 1976	0/15			16	4	8	12	<u>24**</u>	45	4			
6. Hamster, Bayano, 1980	0/4			16	19	16	12	22	<u>26**</u>	4			

\* Reciprocal of geometric mean titer (90% reduction, constant virus-serum dilution PRN test.

\*\* Antisera made in rats.

tions, though most differences detected are not that striking.

Selected PT virus strains, including some considered in these experiments, have been sent to USAMRIID and from there on to Dr. David Bishop at the University of Alabama. They have found minor but reproducible differences in molecular weights of glycoproteins of some strains. Oligonucleotide fingerprints of several strains have also been completed and clear differences were detected between prototype PT virus and isolates from the Bayano and from a man infected in the Darien. Isolates from eastern Panama also killed certain inbred mouse strains. These experiments are continuing.

Chagres viruses: 5 isolates of CHG virus have been selected for testing. These include the prototype strain originally isolated from a man infected in Fort Sherman; 2 other strains isolated in 1979 from soldiers also exposed at Fort Sherman; an isolate from a sentinel hamster exposed in the Bayano in 1976; and an isolate from a pool of sandflies (Lu. sanguinaria) collected in the Bayano also in 1976.

All strains were inoculated subcutaneously into hamsters in an effort to produce single shot immune sera. It soon became apparent, however, that virulence of these strains in hamsters was one means of differentiation, since some strains were highly pathogenic to hamsters while others produced no outward evidence of infection other than seroconversion. Two CHG virus strains did not kill hamsters, while the other 3 strains tested killed nearly all that were infected. When immune hamster sera from strains which did not kill hamsters were tested against all 5 CHG strains, those strains not killing hamsters were found to be antigenically quite similar, while strains which did kill hamsters were antigenically distinct, with one strain (#902921 isolated from L. sanguinaria collected in the Bayano in 1976) virtually non-reactive to our prototype CHG hamster immune sera, though it is reactive with mouse hyperimmune ascitic fluid to prototype CHG virus (Table 7).

When attempts were made to produce immune sera to CHG in rats, all 5 strains failed to initiate infection. We have attempted to infect guinea pigs, but have either been unsuccessful or produced antisera with titers too low to be of value. Our next step will be to attempt to produce monoclonal antibody to these strains of CHG virus. This technology is not available at GML, but may be attempted at USAMRIID.

The results of our preliminary experiments indicate that there is considerable variation among strains of viruses cur-

Table 7 . Hamster survival rates following subcutaneous inoculation with various strains of Chagres virus and results of cross plaque reduction neutralization tests using immune sera (30 day harvest) from surviving hamsters.

<u>Virus</u>	<u>HAMSTER</u> (Surv/Inoc)	<u>ANTISERA</u>			
		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
1. Prototype	5/5	27*	40	<4	NA**
2. Human Ft. Sherman, 1979	5/5	29	48	<4	NA
3. <u>Lutzomyia</u> <u>Bayano, 1976</u>	1/5	<4	<4	32	NA
4. Human Ft. Sherman, 1979	0/5	8	10	<4	NA
5. Hamster Bayano, 1976	0/5	4	5	16	NA

\* Reciprocal of geometric mean titer (90% reduction, constant virus-serum dilution PRN tests).

\*\* NA = Not available.

rently recognized as PT or CHG virus. Such variation can be detected both antigenically and biologically, as demonstrated with the experimental infection of hamsters. The project is now at a stage where it must be addressed using molecular technologies not available at GML. Transfer of the virus strains to USAMRIID will allow that work to continue.

#### E. Conclusions

1. Significant biological differences exist between strains of CHG virus recovered from similar geographic areas as shown by hamster mortality following experimental infection.
2. Significant antigenic differences also exist between strains of CHG virus and preliminary results indicate that antigenic and biologic differences correlate well and suggest 2 distinct subtypes of CHG-like viruses.
3. Antigenic, biologic and molecular differences were also noted among strains of PT viruses, but these differences were not as great as those seen with CHG viruses. Differences detected among strains of PT viruses may represent normal biologic variation.

### VI. Laboratory rearing of phlebotomine sandflies.

#### A. Statement of problem

The purpose of the proposed study was twofold: (1) to improve the conditions which will allow females to go through a second and perhaps third gonotrophic cycle in the laboratory; and (2) to attempt to colonize Panamanian anthropophilic species, especially those species which have been resistant to colonization heretofore (e.g. Lu. trapidoi, Lu. ylephilator, Lu. panamensis.)

#### B. Background

One of the vexing problems of transmission studies of phlebotomine sand fly-borne diseases is the difficulty of establishing and maintaining laboratory colonies of these insects. A few species have been colonized and sustained for periods of time but the majority has defied attempts. The problems of colonization appear to be more serious for tropical species which have narrow limits of tolerance to environmental conditions. Another major obstacle to transmission studies with phlebotomine sandflies is the tendency

of female flies to die after completion of the first gonotrophic cycle.

### C. Approach to problem

The old standard technique to rear phlebotomine sandflies requires using the porous clary pot (Boston bean pot) as rearing vessel, plasterlined vials for oviposition and maintenance of individual adult flies, and standard fine-mesh cages for feeding, mating, maintaining and handling purposes. This technique, with various modifications, has had some success in rearing and colonizing a few species of sand flies. In Panama, for instance, Lu. sanguinaria and Lu. gomezi had been maintained in GML for several years by the use of this standard technique. The major shortcomings of this technique are: (1) it is time-consuming, requiring daily care of colonies; (2) it is moderately productive due to substantial mortality of immature stages caused by fungus and other pathogenic organisms; (3) it fails to prevent female mortality after oviposition; and (4) it is not sufficiently effective to colonize some important vector species.

We proposed to use a different technique to rear and colonize Panamanian anthropophilic species. Instead of the clay pot, plastic or glass jars was used as rearing vessels. Such vessels were used in the past with satisfactory results and with minimum labor expenditure. We preferred small jars to large ones because they can be viewed under a stereoscope, and a small units, losses from disease and infestations can be minimized. Decomposed organic matter and leaf litter from the forest ground (readily available in Panama) was used as a rearing substrate in these jars. Fungus growth in this type of rearing medium is minimal due to naturally occurring bacterial control. The organic matter was supplemented with protein-rich substances, such as liver powder.

Mosquito-Type cages of various sizes with glass or fine nylon mesh in front were used. These cages served many purposes. Rearing vessels were kept in them to prevent escape of adults. Sandflies released in these cages allowed handling and transfer, mating, feeding on blood (by introducing a live host) and sugar, and oviposition.

Sugar feeding is one phase of sandfly maintenance that has been done haphazardly by most workers. We believed it to be very important in the survival of both sexes, in survival of the female after oviposition, and in the development and transmission of parasites. Highly concentrated sugar solutions or solid sugar was provided to sand flies appropriately.

#### D. Results and discussion

Rearing of phlebotomine sandflies started in October 1980 with wild-captured specimens brought to the laboratory in special plaster-lined containers which were protected in styrofoam insulators. Upon arrival at the laboratory, the flies were released in a small cage to be fed. Initially, human arm was introduced into the cage and flies engorged readily. We also provided the flies with anesthetized adult hamsters and guinea pigs. Flies feed on these animals, but not as readily as on the human arm. We found clipping the hair of the belly of these animals induced more flies to feed. Ears and toes were the body areas that flies prefer to feed on. Blood-engorged specimens were then transferred by mouth suction tubes into oviposition vessels. We experimented with a variety of such vessels to determine optimal conditions of oviposition. We used the following: (1) styrofoam cups lined with plaster of paris; (2) plastic vials whose bottoms consist of wet paper or cloth, and (3) a variety of plastic containers with decomposed leaf litter over a layer of wet fine sand. Results indicate that the latter method seems to offer best conditions for survival and oviposition.

We continued our attempts to develop an improved rearing container and concentrated on a number of different plastic containers which contained a layer of moist sand on the bottom to maintain high humidity within the container. The bottom of each container was open and covered with a fine mesh cloth which allowed water to enter into the sand. The sand was then covered with a circular disc of filter paper, over which leaf litter was placed. The moist sand and leaves comprise about 1/4 - 1/3 of the volume of the container. Containers had a small (20-30mm) circular opening on the top, also covered with fine mesh cloth. Drops of liquid sucrose were placed on the screen and served as a sugar source for the sandflies. The overall volume of the container appears to affect the survival of the sandflies, and we have had our best success with containers that were about 120 mm in diameter by 100-140 mm high.

Using sandflies captured in El Aguacate when attracted to human bait, then transported to the laboratory the following morning where they were offered a blood meal on a hamster, we found at best, approximately 20% 5 day survival among fed sandflies. Many experiments have had all flies die before 5 days.

Survival of sandflies in the laboratory remains a severe problem. High mortality, often to 100%, makes any experimental

work very frustrating and productivity very low. This is a very significant problem which will only be overcome through a long term, concerted effort. Improved laboratory maintenance of New World sandflies should be the focus of an independent research effort. Such a project would not necessarily be expensive, only time consuming. Until laboratory survival of sandflies is improved, any attempt to obtain experimental transmission data will be extremely hampered.

#### E. Conclusions

1. An improved sandfly rearing container was designed and tested. The container consisted of a plastic container about 120 mm in diameter by 100-140 mm high. Moist sand covered by filter paper and a layer of leaves was placed inside the container. The bottom of the container was open and covered with fine mesh cloth and the entire container was maintained in a shallow pan of water.

#### F. Recommendations

Laboratory survival of sandflies remains a critical problem. This should be the topic of a long term project.

### **VII. Laboratory transmission studies using phlebotomine sandflies and Phlebotomus fever group viruses.**

#### A. Statement of problem

The objectives of this project were:

1. To document PF group virus transmission to susceptible vertebrate hosts following feeding by infected sandflies.
2. To demonstrate infection per os of "clean" sandflies following feeding on viremic hosts.
3. To demonstrate transovarial transmission of PF group viruses by infected sandflies.

#### B. Background

Phlebotomus fever group viruses have been recovered from a number of different species of New World sandflies. The actual ability of a given species to biologically transmit these viruses has not been demonstrated however, due in part to the difficulty in maintaining sandflies in the laboratory,

especially beyond the first gonotrophic cycle. We hoped to clarify the vectorial competence of suspect vectors through laboratory transmission studies.

#### C. Approach to problem

We began preliminary transmission studies using CHG virus and wild caught adult sandflies. Our first task was to determine the viremia pattern of CHG virus in infected hamsters. We inoculated 5 hamsters subcutaneously with  $7.0 \times 10^3$  pfu of CHG virus. Daily whole blood samples (0.2 ml) were collected and diluted immediately in cell culture maintenance medium (medium 199 with 5% fetal bovine serum), then frozen at  $-70^{\circ}\text{C}$  pending assay. All samples from all hamsters were then assayed simultaneously by the direct plaque technique on Vero cells grown in 96 well panels. Results indicated that hamsters were viremic during days 2 and 3 post-inoculation, with highest titers reached on day 2 ( $10^3$ - $10^4$  pfu/ml). We then experimentally infected hamsters with PT virus following these same procedures and found that they were also viremic on days 2 and 3, with highest titers also on day 2.

For actual transmission studies, we purchased live adult sandflies collected when attracted to human bait in El Agua-cate then transported to the laboratory alive the following day. We arranged to take delivery on Fridays. The preceeding Wednesday, a donor hamster was infected with CHG or PT virus and its belly depilated using a commercially available hair remover (Nair, 8 minutes exposure followed by thorough washing). When the sandflies arrive on Friday mornings, the donor hamster was anesthetized (0.15-0.2 ml of Ketamine) and placed in a cage where the sandflies were allowed to feed to repletion. Following feeding, 0.2 ml of whole blood was drawn and stored at  $-70^{\circ}\text{C}$  for subsequent titration. Engorged sandflies were removed from the feeding cage and placed in the plastic oviposition container described above. At 5 days post-feeding the flies were again allowed to feed, this time on a clean recipient hamster. Recipient hamsters were held for 1 month then bled and examined for antibody by PRN tests.

#### D. Results and discussion

We fed 15 groups of sandflies on hamsters previously inoculated with CHG virus and 12 groups on sandflies previously inoculated with PT virus. Viremia titers were  $3.5 \times 10^3$  -  $1 \times 10^7$  pfu/ml for CHG virus and  $2.6 \times 10^3$  -  $1 \times 10^4$  pfu/ml for PT virus.

Normally between 20-60 sandflies have fed on the viremic hamster form each group. The most abundant species have been Lutzomyia gomezi, Lu. panamensis, Lu sanguinaria, Lu. trapidoi and Lu. ylephilar. Less numerous has been Lu. olmeca. Blood fed individuals were transferred to a holding cage where they were kept 5-7 days, then offered a second blood meal on a susceptible hamster. Generally about 20% of the sandflies survived to take a second bloodmeal. After the second blood feeding, the engorged sandflies were identified and frozen at -70°C pending assay for virus. Eggs deposited following the first bloodmeal have been reared and the F-1 progeny held for virus isolation attempts.

To confirm that blood fed sandflies were ingesting an infectious bloodmeal, 5 individual sandflies were assayed immediately following blood feeding (3 Lu. gomezi, 1 Lu. ylephilar and 1 Lu. olmeca). These flies had fed on a viremic hamster shown to be circulating  $2.6 \times 10^3$  pfu/ml of PT virus. Virus was recovered from all 5 individual flies and confirmed to be PT virus by N tests.

We next assayed 61 individual sandflies which had died 5-7 days following an infectious blood meal. Virus was recovered from a total of 10 (16%) flies which had fed on hamsters viremic with PT virus (5 Lu. gomezi, 2 Lu. panamensis; 2 Lu. sanguinaria and 1 Lu. ylephilar). The virus recovered from each of these flies was confirmed by N test as PT virus.

Virus was recovered from 5 (8%) individual sandflies which had died 5-7 days following feeding on hamsters viremic with CHG virus (3 Lu. ylephilar, 1 Lu. gomezi and 1 Lu. sanguinaria). When N tests were done to confirm the identify of the virus, 4 were shown to be PT virus and only 1 (from Lu. sanguinaria) was CHG virus.

A total of 48 individual sandflies took a second blood meal on 13 PF group virus antibody free hamsters. Sera collected from these hamsters 30 days after being fed upon by these flies all lacked antibody to both CHG and PT virus when assayed by N tests. When these 48 sandflies were assayed individually for the presence of virus, virus was recovered from 71 (15%) flies. Chagres virus was recovered from a Lu. sanguinaria which originally fed on a hamster viremic with CHG virus. Punta Toro virus was recovered from a Lu. olmeca which originally fed on a hamster viremic with PT virus. Punta Toro virus was recovered from the remaining 5 flies (2 Lu. trapidoi, 2 Lu. ylephilar and 1 Lu. panamensis), but all had originally fed on hamster viremic with CHG virus.

A total of 193 F<sub>1</sub> progeny sandflies was reared in the laboratory and assayed individually as newly emerged adults for the presence of virus. From these, 28 (15%) virus isolations were made (Lu. gomezi; 16 positive/129 tested, 12%; Lu. ylephilar, 2/5, 40%; Lu. trapidoi, 9/55, 16%; Lu. panamensis, 1/3, 33% and Lu. sanguinaria, 0/1). All were the progeny of females which had fed on hamsters viremic with CHG virus, with the exception of 28 Lu. gomezi which were from adults which fed upon a PT viremic hamster. All 28 virus isolations were determined to be PT virus by PRN tests, although none of the F<sub>1</sub> Lu. gomezi from adults fed on PT viremic hamsters were positive.

In an attempt to determine if these inconsistent results were due to errors in the identity of our stock virus suspensions, several separate amps of stock virus, both CHG and PT, were identified by N tests. In every case the amps were found to be properly labeled. We next did N tests on all viremic blood samples from donor hamster. Again, all hamsters were circulating the same virus as was inoculated.

In the absence of an obvious laboratory mix-up of seed viruses, the most likely explanation for these inconsistent results is that some of the wild caught adult flies used in the experiments arrived at the laboratory infected with PT virus. Previous field studies in El Aguacate, where the flies were captured, had found infections on the order of 1:600. We had based our use of wild caught adults on the assumption that the likelihood of including infected individuals was quite low, thus when these inconsistent results appeared we were quite surprised. To determine if the field infection rate was much higher than previously reported, we assayed for virus 400 wild caught adults captured from the same area in groups of 10 individuals each. All pools were negative.

It appears then that a possible explanation of these results is that virus infected adult sandflies are not found at a uniform rate throughout the year, but rather are clustered in time, perhaps as a seasonal phenomenon. Transovarial transmission of PT virus by these naturally infected adults could then explain our inconsistent results.

#### E. Conclusions

Results of these experiments were too fragmentary to allow meaningful conclusions to be drawn, with the exception that PT virus was clearly transovarially transmitted by 4 species of sandflies (Lu. gomezi; Lu. ylephilar; Lu. trapidoi and Lu. panamensis.)

**F. Recommendations**

The preliminary results presented in this project clearly indicate that this is a promising topic for further research. Additional investigation appears to be fully justified.

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